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Use of Vetevera Grass *(Chrysopogon zizanioides)* in a Constructed Wetland to Remove Heavy Metals from Kege Wet Coffee Processing Plant, Dale Woreda, Sidama Regional State, Ethiopia

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ABSTRACT: Conventional methods for removing heavy metals from contaminated water are prohibitively expensive and, more significantly, ineffective, especially when the concentration of heavy metals is low. This paper evaluated the use of vetevera grass in a constructed wetland to remove Ca, Cd, Co, Cu, Cr, Fe, K, Mn, Na, Pb, and Zn metals from Kege Wet Coffee Processing Plant, Dale Woreda, Sidama Regional State, Ethiopia using a two vertical flows constructed wetland of 132 square meters in size with Eleven meters in length and 12 meters wide. The 11m * 3m * 1m open space between two constructed wetlands is developed. The second wetland was built, and it serves the same purpose as the previous one but discharges water into the river. The construction of the wetland is performed by digging 20 cm wide, and 30 cm apart furrows. vetiveria grasses was planted at 20 cm intervals. Heavy metals (Ca, Cd, Co, Cu, Cr, Fe, K, Mn, Na, Pb, and Zn) were measured from soil and plant samples from the inlet to the outlet sampling sites using standard procedures from two compartments (soil, and macrophytes) of constructed wetland. Findings indicated that Ca (460.0 ppm) had the highest mean concentration of heavy metals, whereas Ni (0.50 ppm) had the lowest in the soil sample. Metal absorption by vetiver grass is the highest concentration found in plant tissues grown in the following order k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr in shoots. The order of the heavy metal contents in the roots of vetiver grass was k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr. The plant was found to be effective at transferring Mn and Ni from the roots to the shoots based on translocation and bioconcentration, whereas it served as a potential phytostabilizer for Ca, Cu, Cr, Fe, K, Na, and Zn since the TF values are lower than 1, which show that vetiver grass prefers to accumulate heavy metals in the roots rather than the shoot and so supports its potential for phytostabilization. From the present study, it was evident that vetiver grass is an ideal candidate for wastewater treatment using constructed wetland technology.

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Due to the inadequacies of conventional wastewater treatment facilities, wastewater treatment and disposal have been significant environmental challenges in developing countries (Josephat, 2018). Both organic and inorganic components can be attributed to anthropogenic soil and water pollution (Ali and Khan, 2017). The principal inorganic contaminants in wastewater include heavy metals such as chromium,

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manganese, nickel, copper, zinc, cadmium, lead, iron, and arsenic (Siu *et al*., 2007, Barakat, 2011, Khan *et al*., 2011). Heavy metals by definition are metallic elements which have a high atomic weight and much high density at least 5 times that of water, often non-biodegradable and persistent in soils over a long duration (Ali and Khan, 2018). Urban sewage sludge disposal, industrial and agricultural practices, and other human activities release heavy metals into the environment (Khan, 2015). Some heavy metals, like copper, zinc, iron, and manganese, are essential soil micronutrients that living things need in very small amounts for biological metabolism (Pilbeam and Barker, 2007) and other heavy metals, such as Cd, Pb, Cr, Hg, and As, are not essential to the development of living organisms (Abaga *et al*., 2021). Heavy metal contamination of soils and water has become a severe issue that affects soil biomass and causes bioaccumulation via the food chain as a result of metals being transferred from plants to soil (Khan, 2015, Ali *et al*., 2019). Phytoremediation is a technology that transfers pollutants from soils and sediments to the plant tissues without soil structure degradation and soil productivity decrease. The amount of heavy metals taken by plants is influenced by both plant physiology and the amount of metals in the soil (Danielson and Sutherland, 1986). One of the crucial factors in applying the phytoremediation method is choosing the right plant (Seroja *et al*., 2018). Some plant species have a high capacity to accumulate metals in their roots and shoots (Neisi et al., 2014, Gautam and Agrawal, 2017). Researchers have investigated and exploited vetiver in particular for a variety of environmental purposes, including improving water quality, reducing pollution, conserving soil and water, and restoring land (Abaga *et al*., 2021, Mahmoudpour *et al*., 2021). The huge biomass and extensive, 3 m-deep root system of vetiver are its most distinctive characteristics. The vetiver system relies on the use of vetiver grass, which was first identified as having "highly absorbent" characteristics suitable for the treatment of wastewater and leachate produced by landfills (Gupta *et al*., 2012, Banerjee *et al*., 2016). Conventional systems, such as trickling filters and activated sludge, and non-conventional systems, like waste stabilization ponds (WSP) and constructed wetlands, are the two main categories of wastewater treatment methods (Aregu *et al*., 2021). However, there are significant drawbacks to metal removal technology, including high application and maintenance costs, secondary pollution, and challenging operational procedures (Khalid *et al*., 2017, Bolisetty *et al*., 2019). To treat contaminated water, such as coffee wastewater, It is crucial to adopt remediation technology that is affordable, sustainable, eco-friendly, and successful. As a result, phytoremediation is regarded as an inventive, affordable, and ecologically friendly technique for eliminating toxins and hazardous substances from wastewater, such as organic or inorganic pollutants (Antiochia *et al*., 2007, Suelee *et al*., 2017). As, Cd, Cu, Cr, Pb, Hg, Ni, Se, and Zn are just a few of the heavy metals that vetiver is highly tolerant to, demonstrating its distinctive physiological properties (Vargas *et al*., 2016, Suelee *et al*., 2017). Additionally, it is very capable of absorbing nutrients, particularly nitrogen (N) and phosphorus (P), as well as other organic components like biological oxygen demand (BOD) and chemical oxygen demand(COD) (Darajeh *et al*., 2016).

In Africa generally, and in Ethiopia specifically, there is a lack of information about the pollutant removal efficiency of the vetiver grass in a constructed wetland. However, Ethiopian researchers have explored the effective treatment of high-strength wastewater, specifically tannery effluent, utilizing vetiver grass as a constructed wetland plant (Aregu *et al*., 2021). However, the pollutant removal efficiency of the vetiver grass for coffee wastewater quality treatment in this country has not been widely investigated. Previous studies have focused on the uptake of one element by the plant, but in this study, twelve elements were investigated. Also, transfer factors, bio-concentration factors and bioaccumulation factors have been studied. River pollution has become such a concern in Ethiopia as the number of wet coffee refineries grows, so does the amount of trash generated, which is discharged carelessly into neighboring natural waterways that flow into rivers and/or penetrate groundwater, posing a serious threat to surface and groundwater quality (Yemane-Tekle, 2015). This objective of this paper was to evaluate the use of vetevera grass in a constructed wetland to remove Ca, Cd, Co, Cu, Cr, Fe, K, Mn, Na, Pb, and Zn metals from Kege Wet Coffee Processing Plant, Dale Woreda, Sidama Regional State, Ethiopia.

MATERIAL AND METHODS

The study area: Kege wet coffee processing plant, one of the leading coffee-processing plants, is located in Dale Woreda of Sidama Regional State (SRS), near Aposto at the Gidabo River Bridge, at the side of the highway from Addis Ababa to Kenya. The information from the Sidama Region's Environmental Protection Authority indicates that 27,049 tones of the harvested Sidama coffee was exported in 2021/22 while the rest was used for domestic consumption. The range of the average yearly temperature of Dale Woreda is between 9.6°C and 29.2°C.

Constructed wetland unit preparation/ Field Experiment Design: This study is a Randomized controlled trial (RCTs), In this RCTs experiment, the performance of a well-managed constructed wetland performance is tested.A variety of different wetland design and testing methods (either based on volume or area) are available. Each method carries its own set of assumptions, and different equation sets, and they have their strength and weaknesses. Volume-based methods use a hydraulic retention time (HRT) to assess pollutant reduction (Reed et al., 1995) whereas area-based methods assess pollutant reduction using the overall wetland area (Kadlec and Knight, 1996).

Biodegradation of less-degradable pollutants generally requires a combination of anaerobic and aerobic processes. To treat such pollutants with constructed wetlands, therefore, anaerobic and aerobic processes should properly incorporate into wetland systems. Vertical flow constructed wetland systems in which anaerobic and aerobic processes take place sequentially are the most promising options for this purpose (Carballeira *et al*., 2017).

The pond with 8m*8m*1m is constructed for storing wastewater discharged from the coffee processing plant. The pond is used to facilitate the sedimentation process in which heavy solid particles of wastewater are allowed to settle down in the pond. The dimensions of the pond are determined from the daily maximum discharge of wastewater. According to this, during maximum coffee production, 64,000 litres or 64m^3 of wastewater is discharged from the coffee processing plant. Therefore, the sedimentation ponds need to have the capacity of storing this much wastewater per day. That is why the pond is constructed with 8m *8m*1m dimensions as it is shown in Figure 1.

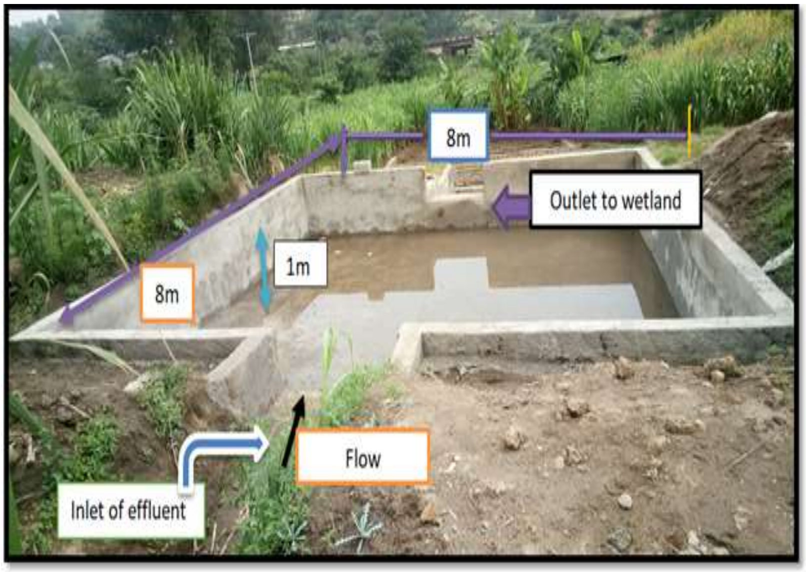

Fig 1: A Constructed sedimentation pond.

The first wetland had a 12m width and 11m length that covered an area of 132 square meters. (Figure 2.A). The design approach used for the Constructed Wetland design of Kege Wet Coffee Processing Plant in the current study is based on hydraulic and organic removal design criteria. In this work, the entire wetland design process mainly followed the criteria given by Kadlec and Knight (1996) and USEPA (2000) for vertical flow-constructed wetland systems.

The construction of the wetland was accomplished by constructing 20cm wide furrows with a spacing of 30cm (Figure 2B). Vetiver grasses have been planted with a spacing of 20cm intervals (Pongthornpruek, 2017). Each pilot unit was filled with soil and sand for plant cultivation to a depth of 60 cm and it was built with a slope of 1% from the inlet towards the outlet zones to prevent backflow (Pongthornpruek, 2017). Water is allowed to flow uniformly via the gravel zone overtopping the masonry wall on the surface of the first vertical flow constructed wetland and then drains down through the filter layer which consists of coarse sand and joins the open water pond downstream underground after passing through the first vetiveria zizanioides plantation. All pilots were planted with vetiver grass (vetiveria zizanioides) for wastewater treatment. The whole system design of the constructed wetland (Figure 3).

Fig 2: First Wetland A) Area of the wetlans, B) Furrows constructed on the wetland

Fig 3: The whole system design of the constructed wetland

Selection of sampling sites, sample collection, transportation and storage: Experimental soils were taken from the soil surface (0 - 20 cm) of constructed wetland (Figure 4) as described by Kassa *et al*., (2002) by using stainless steel soil sampling Auger. Plant samples were also collected in all the sampling sites: S1, S2, S3, S4,S5,S6, S7,S8, S9 and S10 (Figure 4) and rinsed in situ, blotted, pressed, and finally, the samples had been added to non-reacting polyethylene bags, which were then delivered to the laboratory.

Preparation and digestion of samples

A. Preparation and digestion of soil samples from constructed wetland: Any visible plant remnants were removed, and the soil samples were air-dried. The dried soil samples were ground using pestel and mortar, and sieved by using 2 mm nylon sieves. From the total amount of soil samples collected from constructed wetland sites, 500 g of sieved soil, 50 g of which were used for chemical analysis, were produced for each constructed Wetland site. The sieved soil samples were further dried in an oven at 50°C for one and a half hours to make their moisture content uniform. Finally, the samples were stored in sealed polythene and stored in desiccators containing calcium chloride to keep at constant dry weight till digestion. For the digestion of soil samples, the EPA 3050B (Epa US, 1996) method was applied. The procedure used for the digestion of the soil sample was as follows: Initially, 500 mg of the dried and sieved soil sample was added into a digestion vessel. Then 10 mL of a solution prepared by mixing 1:1 ratio of HNO_3 and H_2O (deionized) was added into the vessel and the digestion vessel was taken to the microwave digestion (CEM) adjusted according to the EPA standard for digestion process as described by (Kassa *et al*., 2022) . After digestion is completed, 1 drop of perchloric acid was added to catch the acid and the digestion digestion vessel/tank was removed. Deionized water was added to the digested solution to a final volume of 50 mL

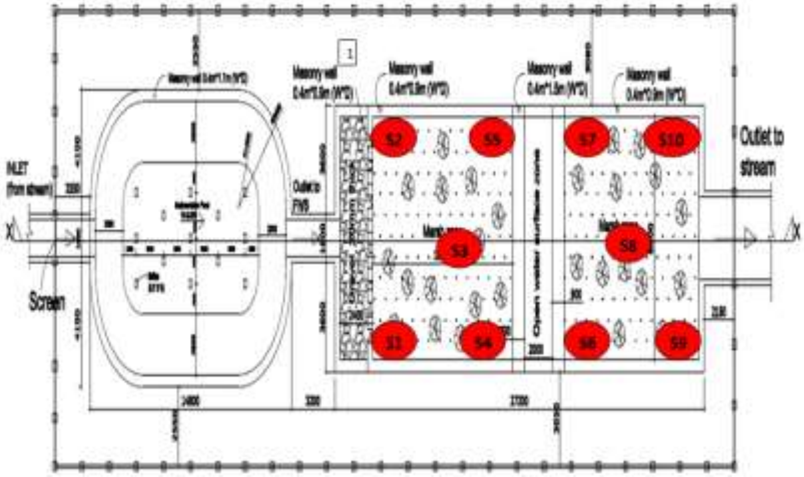

Fig 4: Schematics plan for constructed wetland and sampling sites for plants and soil sample

B .Preparation and plant digestion: Plant samples were collected from ten sampling sites (Figure 4) for plant tissue metal analysis. The plants were manually dug, washed properly with tap water, followed by distilled water to remove adsorbed soil particulates, trimmed carefully to separate root and shoot part of the plant, dry in a direct sunlight for more than 1 month first and finally an oven dry was done at 65°C until constant weight is obtained. From the dry weight of the biomass of each plant tissue, a representative sample was pooled and ground to pass a 100-mesh sieve.

For plant tissue digestion, as described by (Kassa *et al*., 2022) 0.1 g plant tissue sample was pulverized using liquid nitrogen (100 mesh), 8 mL nitric acid was added to the sample and left overnight under Mars digestion (CEM recommended method), and in the next day (preferably after standing pure class), 2 mL 30% hydrogen peroxide (superior grade pure) was added to the plant samples.

Heavy metals analysis: Determination of the metals in the soil and plant samples was made by Flame Atomic Absorption Spectroscopy (FAAS) and Flame Emission Atomic Spectroscopy (FEAS) with an external calibration curve after the parameters such as burner and lamp alignment, slit width and wavelength adjustment was optimized for the maximum signal intensity of the instrument.

For each metal, the respective hollow cathode lamp was inserted into the atomic absorption spectrophotometer, and therefore the solution was successively aspirated into the flame. To avoid loss through ionization, the concentration of Na and K was determined by FEAS. For other metals FAAS were used. Three replicate determinations were carried out for each metal and the same analytical procedure was employed for the determination of elements in blank solutions

*Determination of phytoremediation quotient:*According to a method published by (Baker *et al*., 2000, Shanker *et al*., 2004, Ng *et al*., 2020, Abaga *et al*., 2021), the biological accumulation coefficient (BAC), biological concentration factor (BCF), and translocation factor (TF) were used to evaluate the capacity of vetiver grass for metal accumulation and translocation upwards.

$$\mathrm{BAC} = \frac{CM_{Tillers}}{CM_{soil}} \quad (1)$$

$$\mathrm{BCF} = \frac{CM_{root}}{CM_{soil}} \quad (2)$$

$$\mathrm{BAC} = \frac{CM_{shoot}}{CM_{roots}} \quad (3)$$

Where CMtillers = concentration of heavy metals in tillers; CMroots = concentrations of heavy metals in roots; CMshoot = concentrations of heavy metals in shoots; CMsoil = concentration of heavy metals in soil

Data analysis: The statistical evaluations were carried out using SPSS 24. (SPSS Inc). The Shapiro-Wilk test was used to determine whether the data were normal. The Pearson correlation test was used to assess the association between the heavy metals.

Based on confidence intervals of 95 and 99%, the statistical analyses' significance levels were 0.05 and 0.01, respectively.

RESULTS AND DISCUSSION

Metal concentration in the sediment: The mean metal concentrations and associated information in the soil of the wetland are summarized in Table 1. Based on the mean concentrations, the target elements were arranged in the following descending order in the surface soil of the kege-constructed wetland: Ca> K > Na > Mg > Cu> Fe > Zn > Mn > Ni. Ca had the highest mean concentration of heavy metals (460.0 ppm), whereas Ni had the lowest mean concentrations of heavy metals in soil taken from a constructed wetland (0.50 ppm). The mean metal level found in the soil samples used in this investigation were below the US EPA Soil Quality Guideline (MacDonald and Ingersoll, 2002), and Mean Cd, Cu, Cr, Ni, and Zn concentrations were less than the probable effect concentration (PEC) , which is 3.53 mg/kg, 197 mg/kg, 90 mg/kg, 36 mg/kg, and 315 mg/kg, respectively.

Table 1: Concentration of metals in the soil of Kege constructed wetland (mg/kg).

	Ca	Cd	Co	Cu	Cr	Fe	K	Mn	Na	Ni	Pb	Zn
S1	445.0	-	-	33.0	0.024	10.0	500.0	7.80	150.0	0.70	-	8.90
S2	400.0	-	-	35.0	0.025	11.0	450.0	8.00	165.0	0.66	-	8.50
S3	455.0	-	-	30.0	0.025	9.4	440.0	7.60	145.0	0.80	-	8.00
S4	460.0	-	-	24.0	0.02	9.80	460.0	7.40	167.0	0.75	-	7.50
S5	440.0	-	-	26.0	0.02	11.0	400.0	7.70	180.0	0.65	-	8.90
S6	410.0	-	-	22.0	0.009	9.0	390.0	6.50	175.0	0.64	-	7.20
S7	380.0	-	-	25.0	0.016	10.0	389.0	6.20	160.0	0.61	-	6.70
S8	389.0	-	-	26.0	0.02	8.90	375.0	6.40	167.0	0.59	-	6.40
S9	375.0	-	-	24.0	0.02	9.20	350.0	6.00	172.0	0.55	-	6.20
S10	369.0	-	-	27.0	0.021	8.60	330.0	6.20	163.0	0.50	-	6.00
Mean	412.30	-	-	27.2	0.007	9.69	408.40	6.98	164.4	0.645	-	7.43
± SE	±34.87			±4.185		±0.83	±52.96	±0.78	±10.71	±0.089		±1.11

"-" indicates that the element was not detected.

According to the statistical analysis, there were significant correlations between the concentrations of Ca and K (p, 0.01), Ca and Mn (p, 0.01), Ca and Ni (p, 0.01), and Ca and Zn (p, 0.05). In addition, a correlation was observed for the Cu-Mn, Fe-Mn, and Fe-Zn heavy metal pair (p, 0.05).K-Mn, K-Ni, K-Zn were significanty correlated at (p , 0.05). Similary , there were significant correlations between the concentration of Mn and Ni (p , 0.01), Mn and Zn (p , 0.01), and Ni and Zn (p , 0.05) (Table 2). Previous research found that the primary elements, including Cd, Hg, As, Co, Cu, Ni, Pb, and Cr, were correlated, suggesting that there was a human-made source for the heavy metals (Fu *et al*., 2014, Maanan *et al*., 2015). In this study, similarly, there were a number of paired elements strongly correlated with each other (P 0.01).

Table 2 Pearson correlations between the heavy metals in the soil sampled from the constructed wetland

	Ca	Cu	Fe	K	Mn	Na	Ni	Zn
Ca	1							
Cu	0.20	1						
Fe	0.38	0.48	1					
K	0.79**	0.59	0.54	1				
Mn	0.78**	0.69*	0.73*	0.84**	1			
Na	-0.27	-0.58	0.12	-0.48	-0.27	1		
Ni	0.91**	0.30	0.37	0.83**	0.74**	-0.46	1	
Zn	0.75*	0.59	0.79*	0.81**	0.94**	-0.17	0.68*	1

**Significant coefficient p, 0.05. **Significant coefficient p, 0.01*

Heavy metal contents in shoots of vetiver: The study's findings for the average metal concentration in the vetiver grass shoots under study are shown in Table 3. The concentration of Ca, Cu, Cr, Fe, K and Mn ranged from 48.7 mg/kg to 110 mg/kg, 0.38 mg/kg to 0.913 mg/kg, 0.01 mg/kg to 0.04 mg/kg, 3.0 mg/kg to 6.07 mg/kg, 47.3 mg/kg to 118.3 mg/kg, 3.33 mg/kg to 8.17 mg/kg respectively. Na, Ni and Zn ranged from 37.3mg/kg to 69.7 mg/kg,0.33mg/kg to 0.88 mg/kg and 2.23mg/kg to 4.13 mg/kg respectively, Thus, for all metals, sample site one had the greatest concentration and sample site ten had the lowest concentration. The mean metal concentrations in vetiver grass shoots along sampling sites from the inlet to the outlet did not exhibit a consistent trend (Table 3). The last sample site showed considerably (P 0:05) lower metal concentrations in the vetiver grass, showing that these macrophytes have the capacity to absorb metals and serve as bio-filters for these substances, aiding in the retention of metals in the wetland. Pb and Cd concentrations in vetriver grass were not detected, indicating that there is only a very small amount of these metals in the environment. Metal concentrations in water and soil may have an impact on macrophytes' metal accumulations (Wang *et al*., 2014). Although vetiver grass absorbs metals, plant tissues cultivated in the following order had the highest quantities of metals: K > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr in shoots. Similar findings were made by Banerjee *et al*. (2016) who reported a high concentration of Mn, Fe, Zn, and Cu in the shoot of vetiver, and Gautam and Agrawal (2017) who also revealed a high concentration of Mn, Fe, Zn, and Cu in the shoot of vetiver.

Table 3 Heavy Metal Contents in Shoots of vetiver grass

	Ca	Cu	Cr	Fe	K	Mn	Na	Ni	Zn
S1	110±1.0^{h}	0.913± 0.03^{g}	0.02±0.01^{b}	6.07±0.51^{f}	118.3±3.1^{h}	8.17±1.07^{g}	69.7±5.51^{f}	0.88±0.03h	4.13±0.31^{f}
S2	103.33±2.08^{g}	0.82±0.02^{f}	0.02±0.01^{b}	5.33±0.15^{e}	112.3±2.5^{g}	7.3±0.15^{f}	65.9±0.46^{ef}	0.81±0.02^{g}	3.77±0.25^{e}
S3	88.0±2.64^{f}	0.717±0.2^{e}	0.015±0.01^{a}	5.07±0.12^{e}	94.7±5.13^{f}	7.47±0.25^{f}	62.0±2.65^{de}	0.75±0.02^{f}	3.63±0.15^{de}
S4	81.7±6.65^{def}	0.65±0.01^{cd}	0.01±0.01^{a}	4.67±0.15^{d}	84.3±4.04^{e}	6.80±0.26^{def}	59.3±4.04^{cd}	0.72±0.02^{ef}	3.43±0.15^{d}
S5	87.33±2.52^{ef}	0.69±0.03^{de}	0.01±0.002^{a}	4.63±0.15^{d}	86.0±2.65^{e}	7.10±0.20^{ef}	66.0±2.65^{ef}	0.72±0.02^{f}	3.60±0.10^{d}
S6	81.0±6.56^{de}	0.68±0.02^{de}	0.008±0.002^{a}	4.37±0.15^{cd}	81.0±3.61^{de}	6.50±0.20^{de}	63.0±3.0^{de}	0.69±0.02^{de}	3.30±0.10^{cd}
S7	76.7±2.08^{d}	0.67±0.02^{cde}	0.014±0.002^{a}	4.50±0.10^{cd}	76.7±1.53^{d}	6.30±0.10^{d}	64.3±2.1^{def}	0.65±0.02^{d}	3.33±0.15^{cd}
S8	68.7±1.53^{c}	0.62±0.03^{c}	0.01±0.002^{a}	4.13±0.12^{c}	66.7±2.89^{c}	5.50±0.26^{c}	55.0±2.65^{c}	0.55±0.03^{c}	3.03±0.06^{bc}
S9	57.3±2.52^{b}	0.52±0.03^{b}	0.01±0.002^{a}	3.50±0.30^{b}	57.3±2.52^{b}	4.23±0.25^{b}	44.3±2.52^{b}	0.45±0.02^{b}	2.73±0.21^{b}
S10	46.7±3.05^{a}	0.38±0.08^{a}	0.01±0.002^{a}	3.00±0.20^{a}	47.3±2.52^{a}	3.33±0.15^{a}	37.3±2.52^{a}	0.33±0.02^{a}	2.23±0.16^{a}

Heavy metal contents in roots of vetiver: The order of the heavy metal contents in the roots of vetiver grass was k > Ca > Na > Mn > Fe > Zn > Cu >Ni >Cr (Table 4).The significant accumulation of K and Ca that were found in the root was possibly due to the translocation the metals ion from soils into the root because K and Ca are required macronutrients that are routinely taken by plant for life processes (Mengel and Kirkby, 2001). The observed variance in the amount of metals gathered by vetiver in its various portions suggests that vetiver's ability to absorb metals is mostly reliant on the soil's quality and the concentrations of metals in its natural soil environment (Chunilall *et al*., 2005). The roots accumulated a higher amount of K ,Ca ,Na , Mn , Fe , Zn ,and Cu than the shoots with the exception of Mn and Ni. These results are in agreement with the previous study reported a higher accumulation of metals Fe, Mn, Zn and Cu in roots of vetiver exposed to wastewater (Roongtanakiat *et al*., 2007, Banerjee *et al*., 2019). This shows that vetiver grass can be used as a rhizofiltrator for potassium, calcium, sodium, iron, zinc, and copper due to the greater root absorption of the majority of heavy metals at various metal concentrations (Truong, 2000). Other researchers came to the conclusion that vetiver roots accumulate more heavy metals than the shoot does (Roongtanakiat *et al*., 2007, Pleto *et al*., 2019, Gravand and Hejazi, 2022). In general, vetiver accumulated more heavy metals in its roots than shoots; therefore it is suitable for phytostabilization as suggested by (Yoon *et al*., 2006) and suggested by (Roongtanakiat *et al*., 2008, Roongtanakiat *et al*., 2009). Positive charges on metals allow them to be absorbed into negatively charged areas of root cell walls, leading to greater metal accumulation in roots than in shoots (Yang *et al*., 2005).

Table 4 Heavy metal contents in roots of vetiver grass

		Ca	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
S1	Root	562.7±14.2	40.7±5.92	0.04±0.001	13.0±0.50	567.3±12.5	3.77±0.25	241.3±2.3	0.48±0.08	4.6±0.2
S2	Root	546.7±15.3	33.8±0.15	0.04±0.002	12.0±0.50	558.3±12.6	3.47±0.15	238±7.5	0.38±0.03	4.33±0.15
S3	Root	470±17.3	33.7±0.21	0.03±0.001	11±0.2	447±21	3.33±0.15	200±10	0.31±0.01	3.73±0.15
S4	Root	433.3±15.3	33.5±0.15	0.029±0.00	11±0.2	408±10	3.41±0.15	196.7±5.8	0.32±0.00	3.53±0.06
S5	Root	420.7±10.1	30.7±1.15	0.028±0.00	9.6±0.2	338±8	3.2±0.2	178.3±2.98	0.30±0.01	3.43±0.15
S6	Root	373.3±20.8	9.3±2.52	0.02±0.002	8.43±0.2	361±14.9	3.23±0.15	155±5.0	0.26±0.04	2.9±0.24
S7	Root	374.3±4.01	29.0±3.61	0.02±0.03	8.0±0.0	291±50.9	2.83±0.25	142.7±20.5	0.26±0.02	2.0±0.00
S8	Root	305.7±5.13	22.3±2.52	0.02±0.00	6.3±0.23	216±12.2	2.25±0.05	118.3±7.6	0.21±0.01	1.91±0.04
S9	Root	213.3±15.3	17.7±3.05	0.014±0.00	4.4±0.1	168±10.4	1.90±0.1	112±2.64	0.17±0.01	1.6±0.2
S10	Root	161±6.6	13.7±1.53	0.01±0.002	3.3±0.15	139.7±4.5	1.50±0.05	102±2.66	0.13±0.02	1.45±0.05

Determination of phytoremediation quotient: vetiver potential as a phytoremediation agent can be determined by some index including bio-concentration factor (BCF), bio-accumulation factor (BAC), and translocation factor (TF). The translocation factor and bioaccumulation factor are two indicators of how well plants can remove heavy metals from soil (Baker *et al*., 1994, Dahmani-Muller *et al*., 2000). The bioaccumulation factor calculates the capacity for plants to accumulate heavy metals in various areas of their bodies in relation to the levels of metals in the soil (Branquinho *et al*., 2007). A plant's ability to absorb more metal from the soil is indicated by a BAF value more than 1, while one with a BAF value less than 1 is a metal excluder (Yanqun *et al*., 2005). vetiveria was determined to be a prospective metal excluder rather than a good candidate for the phytoextraction of metals (Ca, Cu, Cr, Fe, K, Na, and Zn) based on BAF values. Previous research corroborated the current study's conclusion that vetiver is a metal-excluder and tolerant plant (Banerjee *et al*., 2016, Gautam and Agrawal, 2017).

Translocation factor measures the plant's potential to translocate heavy metals from roots to the aerial shoots (Baker *et al*., 2000, Shanker *et al*., 2004, Ng *et al*., 2020, Abaga *et al*., 2021). An accumulator has a translocation factor (TF) greater than 1 (Agunbiade *et al*., 2009, Zhang *et al*., 2014). A TF > 1 denotes more metal transfer from the plant's roots to its shoot portion. vetiveria prefers to deposit heavy metal in the root more so than in the shoot, according to a TF value less than 1 (Aksorn and Chitsomboon, 2013). According to the results of the current study, significant amounts of Ca, Cu, Cr, Fe, K, Na, and Zn were absorbed by the roots but were not transported to the shoot system, as shown by TF values < 1 (Table 5). The results of this study are in line with those of Banerjee *et al*. (2016), who found that the Fe, Zn, and Cr contents of vetiver roots were greater than those of the shoots, and with Gautam and Agrawal (2017), who found that vetiver roots absorbed more Fe, Zn, Cu, and Cr than shoots. Long, narrow, waxy leaves and a fibrous root structure are specialized characteristics of vetiver grass that contribute to its ability to tolerate metals. Such specific properties of vetiver limit the transfer of metals via the xylem by reducing evapotranspiration rate (Boonyapookana *et al*., 2005).

Based on the result, the present study revealed that the roots accumulated more heavy metals as the TF values are lower than 1, This confirms vetiver grass' capacity for phytostabilization by showing that it prefers to accumulate heavy metals in the roots rather than the shoot. These results are in agreement with the previous study reported by (Roongtanakiat *et al*., 2007, Banerjee *et al*., 2019, Pleto *et al*., 2019). The fact that the shoots can be utilized for grazing or mulch because there is little heavy metal translocation into them is an important finding (Truong, 2000, Anjum *et al*., 2013).

The manganese had the highest TF of 2.17 and cupper had the lowest with 0.02 at sample site one. The decreased bioavailable percentage of Cu in the soil may be the cause of the low TF for Cu observed in this investigation. For site two, nickel had the highest TF with 2.13 while cupper had the lowest with 0.02. for sample site three, nickel had a translocation factor of 2.42 which was the highest and cupper with only 0.02. The heavy metal nickel had the highest TF of 2.25 and nickel had the lowest with 0.02 for sample site four. For sample site five, nickel had the highest TF with 2.4 while cupper had the lowest with 0.02.

A plant is suitable for phytostabilization or root storage of heavy metals if its TF value is less than 1, and it is suitable for phytoextraction if its TF value is greater 1 (Nabaei and Amooaghaie, 2020). Two distinct types of phytoremediation—phytostabilization and phytoexreaction—involve the application of various functions and traits of plants to remove heavy metals from contaminated soils (Douchichea *et al*., 2012). The main mechanism of phytostabilization is the employment of species of plants that can withstand metals to immobilize heavy metal ions by storing them at the root level without attempting to remove the heavy metals from the upper plant and reduce their bioavailability, preventing their migration into the environment (Marques *et al*., 2009).On the other hand phytoextraction mainly refers to the use of plants to remove contaminants from the environment and concentrate them in above-ground plant tissue(Suman *et al*., 2018). Because of this, phytoextraction entails removing above-ground biomass (shoots) in order to remove heavy metals from polluted soil (Lone *et al*., 2008).

A low TF (TF < 1) was observed for most of the heavy metals considered in this study. However Mn and Ni had high TF(TF > 1) which showed that vetiver grass can be utilized for Mn and Ni phytoextraction based on their remarkably high TF. The behavior of various metals, both antagonistic and synergistic, has a significant impact on the TF values, which in turn affects the uptake and distribution of those metals in plants (Eid and Shaltout, 2014).Mn and Ni more translocation to the shoots may be due to metal sequestration in leaf vacuole and apoplast(Gautam and Agrawal, 2017). On the contrary, Cr had a low TF (TF < 1) in all sample site. The results of this study were consistent with those of the earlier ones, which were TF < 1 (Tariq *et al*., 2016, Chintani *et al*., 2021). The plant's low mobility of Cr from the roots to the shoots may be caused by Cr buildup and saturation in cell vacuoles and apoplast (Park *et al*., 2011, Topcuoglu, 2012). Nickel plays an important role in plants. While it has no toxic effect on plants at low concentrations, nickel is poisonous for plants at high concentrations (Ziarati and Shad, 2017, Naeini and Rad, 2018). Excessive nickel may disturb electron transport chain during photosynthesis and prevent electron establishment and stomatal transactions (Chen *et al*., 2004).

Most vetiver grass sites had BCF values for Cupper metal that were more than one (BCF>1) over the course of the investigation. The majority of BCF results were significantly higher than one, showing that the roots of vetiver plants may store a sizable quantity of Cupper metal. The TF values for calcium metal, on the other hand, were significantly below one (TF>1) throughout the study period. As a result, the research plant is a good phytostabilizer of Cupper metal. This indicates that in the studied plants, the transfer of copper metal from roots to shoots is restricted. This result is consistent with a related study by Pleto *et al*., 2019, which indicated that the roots had the highest concentrations of heavy metals and the shoots had the lowest concentrations.

Table 5 Determination of biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF)

		Ca	**Cu**	**Cr**	**Fe**	**K**	**Mn**	**Na**	**Ni**	**Zn**
S1	BAF	0.25	00.03	0.83	0.61	0.24	1.05	4.5	1.23	0.46
	BCF	1.26	1.23	1.67	1.3	1.13	0.48	1.61	0.69	0.52
	TF	0.20	0.02	0.5	0.47	0.21	**2.17**	0.29	**1.83**	0.90
S2	BAF	0.26	2.34	0.8	0.48	0.25	0.91	0.40	1.23	0.44
	BCF	1.37	0.97	1.6	1.2	1.24	0.43	1.44	0.58	0.51
	TF	0.19	0.02	0.5	0.44	0.2	**2.1**	0.28	**2.13**	0.87
S3	BAF	0.19	0.02	0.6	0.54	0.22	0.98	0.43	0.94	0.45
	BCF	1.03	1.12	1.2	1.20	1.02	0.44	1.38	0.39	0.47
	TF	0.19	0.02	0.5	0.46	0.21	2.24	0.31	2.42	0.97
S4	BAF	0.18	0.03	0.5	0.48	0.18	0.92	0.36	0.96	0.46
	BCF	1.04	1.40	1.45	1.12	0.89	0.46	1.18	0.43	0.47
	TF	0.19	0.02	0.35	0.42	0.21	2.0	0.30	**2.25**	0.97
S5	BAF	0.2	0.03	0.5	0.40	0.215	0.92	0.37	1.11	0.40
	BCF	1.05	1.2	1.4	0.87	0.845	0.42	0.98	0.46	0.39
	TF	0.21	0.02	0.36	0.48	0.25	**2.2**	0.37	**2.4**	1.01
S6	BAF	0.2	0.03	0.89	0.40	0.20	1	0.36	1.08	0.46
	BCF	0.91	1.33	2.2	0.77	0.90	0.5	0.89	0.41	0.40
	TF	0.22	0.02	0.4	0.52	0.22	**2.01**	0.41	**2.65**	1.14
S7	BAF	0.2	0.03	0.88	0.41	0.19	1.01	0.40	1.07	0.5
	BCF	1.0	1.2	1.25	0.73	0.73	0.46	0.89	0.43	0.30
	TF	0.21	0.02	0.7	0.56	0.26	2.23	0.45	**2.5**	1.65
S8	BAF	0.18	0.02	0.5	0.46	0.17	0.86	0.33	0.93	0.47
	BCF	0.79	1.00	1	0.71	0.54	0.35	0.71	0.36	0.30
	TF	0.22	0.03	0.5	0.66	0.31	**2.44**	0.47	**2.62**	1.57
S9	BAF	0.15	0.02	0.5	0.38	0.14	0.71	0.26	0.82	0.44
	BCF	0.57	0.75	0.7	0.48	0.42	0.31	0.65	0.31	0.26
	TF	0.27	0.03	0.71	0.79	0.34	2.23	0.39	**2.65**	1.71
S10	BAF	0.13	0.01	0.5	0.35	0.12	0.54	0.23	0.66	0.37
	BCF	0.44	0.5	0.5	0.38	0.35	0.24	0.63	0.26	0.24
	TF	0.29	0.03	0.99	1.1	0.34	2.22	0.36	2.54	1.54

Conclusions: The effluent from the coffee processing factory can potentially be cleaned up very well using the vetiver grass system. According to the findings, heavy metals had accumulated on roots and shoots. The vetiver grass absorbed harmful heavy metals like nickel, chromium, manganese, and copper. Based on the calculated translocation factor, the vetiver grass preferred to accumulate heavy metals in the roots. The vetiver grass system is a relatively inexpensive technology with a significant potential benefit for reducing soil contamination. Based on metal translocation and bio concentration factors, vetiver behaved as a phytostabilizer for all the heavy metals (Ca, Cu, Cr, Fe, K, Na and Zn) and efficient in translocation factor (TF > 1), of Mn and Ni from roots to shoot, serving as a good phytoextractor. We recommended that a follow-up investigation be carried out at a different season.

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